Bayer HealthCare

QC

CE

HAV IgM (aHAVM)

Contents

REF	Contents
05004800	2 vials of Negative Control CONTROL ⊖
	2 vials of Positive Control CONTROL ⊕
	Expected Values Card and barcode labels

Preliminary 00371260 Rev. A, 2004-06

Intended Use

For *in vitro* diagnostic use in monitoring the performance of the HAV IgM assay on the ADVIA Centaur® Systems. The performance of the HAV IgM quality control material has not been established with any other anti-HAV IgM assays.

Control Description

Volume	Ingredients	Storage	Stability
7.0 mL/vial	Processed human plasma negative and positive for IgM antibodies to HAV with preservatives	2–8°C	Until the expiration date on the vial label or onboard–8 hours

R43 **Irritant!** May cause sensitization by skin contact. Avoid contact with skin.
S24, S37 Wear suitable gloves. **Contains:** 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one

CAUTION! POTENTIAL BIOHAZARD: The controls contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[1-3] Use eye protection and gloves when handling this product; wash hands after handling.

The negative control has been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2. The positive control contains human plasma that is reactive for anti-HAV IgM. The units were treated with a BPL-UV inactivation procedure, however, all products manufactured using human source material should be handled as potentially infectious.

For *In Vitro* Diagnostic Use.

Preparing the Quality Control Material

Gently swirl and invert the vials to ensure homogeneity.

Using the Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the HAV IgM quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HAV IgM assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 4 to 5 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Reviewing, Editing, and Printing Results

For detailed information about reviewing, editing, and printing quality control results, refer to the system operating instructions or to the online help system.

Expected Results

Refer to the *Expected Values* card for the assigned values specific for the lot number of the HAV IgM quality control material. The expected values are traceable to the standardization of the HAV IgM assay. For additional information, refer to the reagent instructions for use.

The expected values should be used only as a guide in evaluating performance. Since performance is subject to the design and condition of each instrument or reagent system, it is recommended that each laboratory establish its own expected values and acceptable limits. The mean values established should fall within the range specified in *Expected Values*. Individual results may fall outside the range.

Taking Corrective Action

If the quality control results do not fall within the suggested *Expected Values* or within the laboratory's established values, then do the following:

- consider the sample results invalid and repeat testing if controls are out of range
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary contact Bayer HealthCare for more assistance

Limitations

The results obtained using the HAV IgM quality control material depend on several factors. Erroneous results can occur from improper storage, inadequate mixing, or sample handling errors associated with system or assay procedures.

- Do not return any quality control materials back into the vials after testing because evaporation and contamination can occur, which may affect results.
- Dispose of any quality control material remaining in the sample cups after 8 hours.
- Do not refill sample cups when the contents are depleted. If required, dispense fresh quality control materials.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. National Committee for Clinical Laboratory Standards. Procedures for the Handling and Processing of Blood Specimens; Approved guideline-2nd Edition. NCCLS document H18-A2. Wayne (PA):NCCLS;1999.
2. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8.
3. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue; approved guideline. NCCLS Document M29-A2. Wayne (PA):NCCLS;2001.

HAV IgM (aHAVM)

Assay for the Detection of IgM Antibodies to Hepatitis A Virus

Assay Summary

Sample Type	*Serum, potassium EDTA plasma, lithium or sodium heparinized plasma*
Sample Volume	*20 µL*
Calibrator	*HAV IgM*

Contents

REF	Contents	Number of Tests
05004126	1 ReadyPack® primary reagent pack containing ADVIA Centaur® HAV IgM Lite Reagent, Solid Phase, and Ancillary Well Reagent 1 Ancillary pack containing ADVIA Centaur HAV IgM Ancillary Reagent ANC ADVIA Centaur HAV IgM Master Curve card 1 vial HAV IgM Low Calibrator CAL L 1 vial HAV IgM High Calibrator CAL M ADVIA Centaur HAV IgM Calibrator Assigned Value card	100

For a definition of symbols used in product labeling, please refer to Appendix D, *Understanding the Symbols*, in the *ADVIA Centaur® Assay Manual.*

Intended Use

The ADVIA Centaur® HAV IgM assay is an *in vitro* diagnostic immunoassay for the qualitative determination of IgM response to the hepatitis A virus (HAV) in human serum or plasma (potassium EDTA, lithium or sodium heparinized) using the ADVIA Centaur System. This assay is intended for use as an aid in the diagnosis of acute or recent infection (usually 6 months or less) with hepatitis A virus.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, infants, or children.

WARNING: This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

United States federal law restricts this device to sale by or on the order of a physician.

Materials Required But Not Provided

REF	Description	Contents
	ADVIA Centaur System	
05004800	ADVIA Centaur HAV IgM quality control material	2 x 7.0 mL Negative Control CONTROL 2 x 7.0 mL Positive Control CONTROL Expected Value card
07948423 (110314)	ADVIA Centaur Multi-Diluent 2* M-DIL 2	2 ReadyPack ancillary reagent packs containing 10 mL/pack
01137199 (112351)	ADVIA Centaur Wash 1 WASH 1	2 x 1500 mL/pack

*A minimum of two ADVIA Centaur Multi-Diluent 2 ancillary reagent packs are required for each ADVIA Centaur HAV IgM primary reagent pack (100 tests).

Summary and Explanation of the Test

The ADVIA Centaur HAV IgM assay is an antibody capture microparticle chemiluminometric immunoassay used for the detection of IgM antibody to hepatitis A virus in human serum or plasma.

Hepatitis A is caused by infection with the hepatitis A virus. HAV is a 27 nanometer single-stranded, nonenveloped, RNA virus that is classified as a picornavirus. Transmission of hepatitis A is usually via the fecal-oral route and infection occurs mainly due to contaminated food or poor sanitary conditions.[1,2]

Hepatitis A virus replicates in the liver. The virus is excreted in the bile and shed in the stool. Only one serotype has been observed among HAV isolates collected from various parts of the world. The average incubation period for HAV infection is 30 days with a range of 15 to 40 days. Chronic infection has not been reported to occur following HAV infection. Symptoms last approximately 2 weeks and include hepatomegaly, jaundice, dark urine, fatigue, and gastrointestinal distress such as anorexia, nausea, vomiting, and abdominal pain. At the onset of symptoms resulting from HAV infection, antibody to HAV is detectable. The early antibody response is largely comprised of the IgM antibody subclass. Anti-HAV IgM is detectable usually for 3 to 6 months after the onset of illness, whereas anti-HAV IgG can persist indefinitely. Because of the transient production of anti-HAV IgM, its presence in sera indicates ongoing or recent infection and is the most useful serological marker for diagnosing acute HAV infection.[1-4]

Since symptomatic hepatitis A viral infections can not be clinically distinguished from hepatitis B or C viral infections, serological testing is important for proper diagnosis.

Assay Principle

The ADVIA Centaur HAV IgM assay is an IgM capture immunoassay using a 2-pass format. In the first pass the sample is diluted using Multi-Diluent 2. After sample dilution biotinylated anti-human IgM monoclonal antibody is added to the cuvette binding IgM from the diluted patient sample. The IgM complex is then captured by the addition of streptavidin coated magnetic latex particles (MLP). The IgM-MLP is washed and resuspended.

In the second pass the anti-HAV IgM captured on the Solid Phase is detected by the

sequential addition of HAV antigen and acridinium ester-labeled mouse anti-HAV antibody.

The system automatically performs the following steps:

- dispenses 20 µL of sample and 180 µL of Multi-Diluent 2 into a cuvette
- aspirates 60 µL of diluted sample and dispenses it into a cuvette
- dispenses 150 µL of Ancillary Reagent and incubates for 6 minutes at 37°C
- dispenses 150 µL of Solid Phase and incubates for 18 minutes at 37°C
- separates the Solid Phase from the mixture and aspirates the unbound reagent
- washes the cuvette with Wash 1
- resuspends the particles in 250 µL of Wash 1 and incubates for 6.75 minutes at 37°C
- dispenses 50 µL each of Ancillary Well Reagent and Lite Reagent and incubates for 18 minutes at 37°C
- separates the Solid Phase from the mixture and aspirates the unbound reagent
- washes the cuvette with Wash 1
- dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system

The relative light units (RLUs) detected by the ADVIA Centaur System are used to calculate the Signal-to-Cutoff (S/CO) Value from the Master Curve. A result of reactive or nonreactive is determined according to the S/CO Value established with the calibrators. Refer to *Interpretation of Results* for a description of the Cutoff Value calculation.

Specimen Collection and Handling

Serum, potassium EDTA plasma, lithium or sodium heparinized plasma are the only recommended sample types for this assay.

Heparin has been shown to increase the S/CO values in some HAV IgM reactive samples by up to 14% relative to serum. Equivocal and reactive heparin plasma samples near the cutoff should be interpreted accordingly.

Do not use specimens with obvious microbial contamination. The performance of the ADVIA Centaur HAV IgM assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma such as saliva, urine, amniotic fluid, or pleural fluid.

The following general recommendations for handling and storing blood samples are furnished by the National Committee for Clinical Laboratory Standards,[5] and augmented with additional sample handling studies using the ADVIA Centaur HAV IgM assay:

- Handle all samples as if capable of transmitting disease.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells. Centrifuge samples within 2

hours post draw.

- Test samples as soon as possible after collecting. Store samples at 2 to 8°C if not tested within 8 hours of collection.
- Store samples stoppered and upright at all times at 2 to 8°C up to 2 days.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage. Samples may be stored at or below -20°C for up to 180 days. Do not store in a frost-free freezer. When specimens are subjected to up to 4 freeze/thaw cycles, no qualitative differences are observed.Thoroughly mix thawed samples and centrifuge at 10,000g for 2 minutes before using. Collect the supernatant into a clean vial.
- Package and label samples for shipment in compliance with applicable federal and international regulations covering the transport of clinical samples and etiological agents. Store samples stoppered and upright at 2 to 8°C upon arrival. If shipment is expected to exceed 2 days, ship specimens frozen.

Before placing samples on the system, ensure the following:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation (example: 1500xg for 10 minutes; follow tube manufacturer's recommendations).
- Samples are free of bubbles or foam.

Reagents

Store the reagents upright at 2–8°C.

Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents*, in the *ADVIA Centaur® Assay Manual.*

Protect from sunlight.

Protect reagent packs from all light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2–8°C away from light sources.

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIACentaur HAV IgM Primary Reagent ReadyPack	Lite Reagent	5.0 mL/ reagent pack	anti-HAV mouse monoclonal antibody[1] (~0.200 µg/mL) labeled with acridinium ester in buffer with bovine serum albumin, surfactant, sodium azide (<0.1%), and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Solid Phase	15.0 mL/ reagent pack	streptavidin coated paramagnetic microparticles in buffer with bovine serum albumin, surfactant, sodium azide (<0.1%), and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
	Ancillary Well Reagent	5.0 mL/ reagent pack	inactivated purified hepatitis A virus (<0.1 µg/mL) in buffer with bovine serum albumin, surfactant, sodium azide (<0.1%), and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
ADVIACentaur HAV IgM ANC Ancillary Reagent ReadyPack	Ancillary Reagent	25.0 mL/ reagent pack	biotinylated monoclonal mouse anti-human IgM (~0.500 µg/mL) in buffer with bovine serum albumin, mouse IgG, surfactant, sodium azide (<0.1%), and preservatives	2-8°C	until the expiration date on the pack label. For onboard stability, refer to *Onboard Stability and Calibration Interval.*
HAV IgM calibrator vials	Calibrators	2.0 mL/ vial	processed human plasma positive for IgM antibodies to HAV with preservatives	2-8°C	until the expiration date on the vial or onboard 8 hours
HAV IgM quality control material vials[2]	Controls	7.0 mL/ vial	processed human plasma negative and positive for IgM antibodies to HAV, with preservatives	2-8°C	until the expiration date on the vial or onboard 8 hours
ADVIA Centaur M-DIL 2 ancillary reagent Readypack *	Multi-Diluent 2	10.0 mL/ reagent pack	goat serum with sodium azide (0.1%) and preservatives	2-8°C	until the expiration date on the pack label or 28 consecutive days after accessing the ancillary reagent pack
ADVIACentaur WASH 1[2]	Wash 1	1500 mL/ pack	phosphate buffered saline with sodium azide (<0.1%) and surfactant	2-25°C	until the expiration date on the vial or onboard 14 days

1. The antibody recognizes a conformational epitope on the assembled hepatitis A virus.
2. See *Materials Required But Not Provided.*

Precautions and Warnings

For *In Vitro Diagnostic* Use.

CAUTION: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

R43 **Irritant!** May cause sensitization by skin contact. Avoid contact with skin. Wear suitable gloves.
S24 **Contains:** 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one; included in
S37 Calibrators and Controls.

CAUTION! POTENTIAL BIOHAZARD: Some components of this product contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[6-8]

The negative control has been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2. The positive control and calibrators contain human plasma that is reactive for anti-HAV IgM. The units were inactivated using a BPL-UV inactivation procedure. The Ancillary Well Reagent contains HAV virus inactivated with formalin. All products manufactured using human source material should be handled as potentially infectious.

Loading Reagents

Ensure that the system has sufficient primary and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

CAUTION: Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents,* in the *ADVIA Centaur Assay Manual.*

Load the ReadyPack primary reagent packs in the primary reagent compartment using the arrows on the packs as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. Load the Multi-Diluent 2 ancillary reagent pack in the ancillary reagent entry. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

CAUTION: The Low and High Calibrators provided in this kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

CAUTION: The Ancillary Reagent provided in this kit is matched to the Lite Reagent, Solid Phase, and Ancillary Well Reagent. Do not mix Ancillary Reagent lots with different lots of Lite Reagent, Solid Phase, and Ancillary Well Reagent.

NOTE: The Ancillary Reagent pack contains more volume than required to perform 100 tests. Since the Ancillary Reagent is matched to the Lite Reagent, Solid Phase, and Ancillary Well Reagent in the ReadyPack primary reagent pack, discard the Ancillary Reagent pack when the ReadyPack primary reagent pack is discarded. Do not use beyond the onboard stability.

Onboard Stability and Calibration Interval

Onboard Stability	Calibration Interval
41 days	28 days

Additionally, the ADVIA Centaur HAV IgM assay requires a two-point calibration:

- when changing lot numbers of primary reagent packs
- when replacing system components
- when quality control results are repeatedly out of range

CAUTION:

- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

Master Curve Calibration

The ADVIA Centaur HAV IgM assay requires a Master Curve calibration when using a new lot number of Lite Reagent, Solid Phase, and Ancillary Well Reagent. For each new lot number of Lite Reagent, Solid Phase, and Ancillary Well Reagent, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

Calibration

For calibration of the ADVIA Centaur HAV IgM assay, use ADVIA Centaur HAV IgM Calibrators provided with each kit. The calibrators provided in this kit are matched to the ReadyPack primary reagent pack.

Using Barcode Labels

NOTE: Calibrator barcode labels are lot number specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur HAV IgM Calibrator barcode labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur HAV IgM assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard. For detailed information about entering calibrator values, refer to the system operating instructions or to the online help system.

NOTE: This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.

1. Schedule the calibrators to the worklist.
2. Label two sample cups with calibrator barcode labels: one for the low and another for the high.

NOTE: Each drop from the calibrator vial is approximately 50 µL.

3. Gently mix the Low and High Calibrators and dispense at least 4 to 5 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any calibrator remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Quality Control

For quality control of the ADVIA Centaur HAV IgM assay, use ADVIA Centaur HAV IgM quality control material. Refer to the Expected Value card for the suggested expected values specific for the lot number of the positive and negative controls. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTE: The quality control material furnished is intended to monitor substantial reagent failure. If additional controls are desired, it is recommended to run a negative control and a positive control close to the clinically relevant point. Further, the quality control material furnished is in a serum matrix. It may not adequately control the assay for plasma specimens. The user should provide alternate control material for plasma matrix.

Using Barcode Labels

NOTE: Control barcode labels are lot number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur HAV IgM quality control barcode labels to identify the positive and negative sample cups when performing the ADVIA Centaur HAV IgM assay. Place the barcode label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each workshift that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label two sample cups with quality control barcode labels: one for the positive, and another for the negative.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 4 to 5 drops into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Taking Corrective Action

If the quality control results do not fall within the suggested Expected Values or within the laboratory's established values, then do the following:

- consider the sample results invalid and repeat testing if controls are out of range.
- investigate and determine the cause for the unacceptable control results.
- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer HealthCare.
- verify that the materials are not expired.
- verify that required maintenance was performed.
- if necessary contact Bayer HealthCare for more assistance.
- when the condition is corrected, retest the controls and confirm that results are within acceptable limits.
- it is advisable to repeat some or all patient specimens before reporting results for this run.

Sample Volume

This assay requires 20 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the *ADVIA Centaur Reference Manual.*

Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

CAUTION: Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Prepare the sample container for each sample, and place barcode labels on the sample containers, as required.
2. Load each sample container into a rack, ensuring that the barcode labels are clearly visible.

3. Place the racks in the entry queue.
4. Ensure that the assay reagents are loaded.
5. Start the entry queue, if required.

Procedural Notes

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Interpretation of Results

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

The system reports HAV IgM results in S/CO Values and as reactive, equivocal, or nonreactive.

- Samples with a calculated value of less than 0.80 S/CO Value are considered nonreactive for IgM antibodies to hepatitis A virus.
- Samples with a calculated value greater than or equal to 0.80 S/CO Value and less than 1.20 S/CO Value are considered equivocal and must be repeated. It is recommended that the test be repeated in duplicate and the results be reported based on the repeat results. If the results are still equivocal or uninterpretable after repeat testing, obtain a new specimen and retest using the ADVIA Centaur HAV IgM assay.
- Samples with a calculated value greater than or equal to 1.20 S/CO Value are considered reactive for IgM antibodies to hepatitis A virus.
- The cutoff for the ADVIA Centaur HAV IgM assay was verified based on results of Receiver-Operator characteristics (ROC) Curve[9] and clinical agreement generated from the clinical studies.
- The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present.
- Sample results are invalid and must be repeated if the controls are out of range.

CAUTION: Heparin has been shown to increase the S/CO values in some HAV IgM reactive samples by up to 14% relative to serum. Equivocal and reactive heparin plasma samples near the cutoff should be interpreted accordingly.

Limitations

- The ADVIA Centaur HAV IgM assay is limited to the detection of IgM antibodies to hepatitis A virus in human serum or plasma (potassium EDTA plasma, lithium or sodium heparinized plasma).
- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to hepatitis A virus.
- The ADVIA Centaur HAV IgM assay can be used to determine if a patient has or

recently had an acute or asymptomatic hepatitis A infection. This test does not measure anti-HAV IgG and therefore cannot be used to determine a patient's immune status to hepatitis A.

- The calculated values for hepatitis A in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with different assay methods cannot be used interchangeably.
- Assay performance characteristics have not been established for immunocompromised, immunosuppressed, infants, children, or adolescent patients.
- The performance of the ADVIA Centaur HAV IgM assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic fluid, or pleural fluid.
- Do not use specimens with obvious microbial contamination.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[10] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- In patients receiving therapy with high doses of biotin (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
- A reactive HAV IgM result does not exclude co-infection by another hepatitis virus.

Expected Results

The prospective study population for the ADVIA Centaur HAV IgM assay consisted of 846 patients. Of these 846 patients, 249 patients (29.43%) were from the high risk population, 178 patients (21.04%) were from the signs and symptoms population, 2 patients (0.24%) were from the acute HAV infected patient population, 215 patients (25.41%) were from the HAV infected/HAV recovered patient population and 202 patients (23.88%) were from the hospitalized patient population. The prospective study population was 29.20% Caucasian, 37.59% Hispanic, 28.37% Black, 1.65% Asian, and 3.2% from unknown or other ethnicity. The majority of patients were male (58.16% male and 41.84% female). The mean age was 48.42 years (range of 18 to 101 years). Patients in the prospective study population were from the following geographic regions: Florida (58.39%), Texas (29.67%), and New York (11.94%).

The ADVIA Centaur HAV IgM results for the prospective population for all sites combined by age group and gender are summarized in the following table:

ADVIA Centaur HAV IgM Assay Distribution of Prospective Population by Age Group and Gender; All Testing Sites

		Reactive		Equivocal		Non-reactive		Total	
Age (years)	Gender	N	%	N	%	N	%	N	%
10-19	Female	0		0		2	100.00	2	40.00
	Male	0		0		3	100.00	3	60.00
	Overall	0		0		5	100.00	5	100.00
20-29	Female	0		0		36	100.00	36	59.02
	Male	0		0		25	100.00	25	40.98
	Overall	0		0		61	100.00	61	100.00
30-39	Female	0		0		71	100.00	71	47.02
	Male	0		0		80	100.00	80	52.98
	Overall	0		0		151	100.00	151	100.00
40-49	Female	0		0		93	100.00	93	35.63
	Male	0		1	0.60	167	99.40	168	64.37
	Overall	0		1	0.38	260	99.62	261	100.00
50-59	Female	0		0		75	100.00	75	36.76
	Male	0		1	0.78	128	99.22	129	63.24
	Overall	0		1	0.49	203	99.51	204	100.00
60-69	Female	0		0		45	100.00	45	45.92
	Male	0		0		53	100.00	53	54.08
	Overall	0		0		98	100.00	98	100.00
70+	Female	1	3.13	0		31	96.88	32	48.48
	Male	0		0		34	100.00	34	51.52
	Overall	1	1.52	0		65	98.48	66	100.00
Total	Female	1	0.28	0		353	99.72	354	41.84
	Male	0		2	3.44	490	99.59	492	58.16
	Overall	1	0.12	2	0.24	843	99.65	846	100.00

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[11]

Performance Characteristics

The prospective study population for the ADVIA Centaur HAV IgM assay consisted of 846 patients. Of these 846 patients, 249 patients (29.43%) were from high risk population, 178 patients (21.04%) were from the signs and symptoms of hepatitis population, 2 patients (0.24%) were from the acute HAV infected population, 215 patients (25.41%) were from the HAV infected/HAV recovered patient population, and 202 patients (23.88%) were from the clinic/hospitalized patient population. The prospective study population was 29.20% Caucasian, 37.59% Hispanic, 28.37% Black, 1.65% Asian, and 3.2% from unknown or other ethnicity. The majority of patients were male (58.16% male and 41.84% female). The mean age was 48.42 years (range of 18 to 101 years) Patients in the prospective study population were from the following geographic regions: Florida (58.39%), Texas (29.67%), and New York (11.94%).

Comparison of Results

In addition to the 846 prospective samples, 103 samples were obtained retrospectively for the HAV acute population. The total of 949 samples were run using the ADVIA Centaur HAV IgM assay and a comparative anti-HAV IgM assay for each subject category. The following results were obtained:

Comparison of Results in High Risk, Signs and Symptoms, Clinic/Hospitalized, and HAV Infected Populations ADVIA Centaur HAV IgM Assay versus Comparative Anti-HAV IgM Assay (All Testing Sites)

	Comparative Anti-HAV IgM Assay						*Comparative Anti-HAV IgM Assay*						*Comparative Anti-HAV IgM Assay*						
	Negative						*Equivocal*						*Positive*						
Subject Category	*ADVIA Centaur HAV IgM Assay*						*ADVIA Centaur HAV IgM Assay*						*ADVIA Centaur HAV IgM Assay*						
	Reactive		*Nonreactive*		*Equivocal* [1]		*Reactive*		*Nonreactive*		*Equivocal* [1]		*Reactive*		*Nonreactive*		*Equivocal* [1]		*Total*	
	N	*(%)*	*N*	*(%)*	*N*	*(%)*	*N*	*(%)*	*N*	*(%)*	*N*	*(%)*	*N*	*(%)*	*N*	*(%)*	*N*	*(%)*	*N*	*(%)*
Acute	0	0.00	1	0.95	0	0.00	0	0.00	2	1.90	0	0.00	99	94.29	1	0.95	2	1.90	105	11.06
Clinic / hospitalized	0	0.00	200	99.00	1	0.50	0	0.00	0	0.00	0	0.00	1	0.50	0	0.00	0	0.00	202	21.29
High Risk	0	0.00	248	99.60	0	0.00	0	0.00	1	0.40	0	0.00	0	0.00	0	0.00	0	0.00	249	26.24
Infected / Recovered	0	0.00	214	99.53	0	0.00	0	0.00	1	0.47	0	0.00	0	0.00	0	0.00	0	0.00	215	22.66
Signs / Symptoms	0	0.00	176	98.88	1	0.56	0	0.00	1	0.56	0	0.00	0	0.00	0	0.00	0	0.00	178	18.76
Total	0	0.00	839	88.41	2	0.21	0	0.00	5	0.53	0	0.00	100	10.54	1	0.11	2	0.21	949	100.00

1 Equivocal results following repeat testing

Percent Agreement

For purposes of percent agreement calculations, the ADVIA Centaur equivocal results (n = 4) were assigned the opposite clinical interpretation than that of the comparative assay result and analysis performed. Comparative assay equivocals (n = 5) were removed from the analysis. The percent agreement between the ADVIA Centaur HAV IgM assay and the comparative HAV IgM assay by subject category across all testing sites is summarized in the following table:

ADVIA Centaur HAV IgM Assay
Percent Agreement and Confidence Intervals in All Subject Categories
ADVIA Centaur HAV IgM Assay vs. HAV IgM Comparative assay
All Testing Sites[a]

Subject Category	Positive Percent Agreement % (x/n)[b]	95% Exact Confidence Interval	Negative Percent Agreement % (x/n)[c]	95% Exact Confidence Interval
Acute	97.06 (99/102)	91.64 to 99.39	100.00 (1/1)	2.50 to 100.00
Clinic / Hospitalized	100.00 (1/1)	2.50 to 100.00	99.50 (200/201)	97.26 to 99.99
High Risk	0.00 (0/0)	NA	100.00 (248/248)	98.52 to 100.00
Infected / Recovered	0.00 (0/0)	NA	100.00 (214/214)	98.29 to 100.00
Signs / Symptoms	0.00 (0/0)	NA	99.44 (176/177)	96.89 to 99.99
Total	97.09 (100/103)	91.72 to 99.40	99.76 (839/841)	99.14 to 99.97

a *Five samples were excluded from the percent agreement analysis. All five samples were Comparative assay equivocal and ADVIA Centaur HAV IgM negative*
b *x = the number of ADVIA Centaur HAV IgM results that are reactive in agreement with the comparative HAV IgM; n = the total number of comparative HAV IgM results that are reactive*
c *x = the number of ADVIA Centaur HAV IgM results that are nonreactive in agreement with the comparative HAV IgM; n = the total number of comparative HAV IgM results that are nonreactive*

Seroconversion Panels

Four commercially available HAV patient seroconversion panels were tested in the clinical sites using the ADVIA Centaur HAV IgM assay to determine the seroconversion sensitivity of the assay. The performance of the ADVIA Centaur HAV IgM assay on the seroconversion panels closely matched the performance of the comparative assay. The following results were obtained:

	Anti-HAV IgM Positive Result From Initial Draw Date		Comparative assay vs ADVIA Centaur Assay
Panel ID	Comparative assay (Days)	ADVIA Centaur Assay (Days)	Difference (Bleeds)*
RP004	7	7	0
RP013	9	9	0
PHT902	16	16	0
ProMedx 1	1	1	0

* The difference in bleed numbers is relative to the comparative assay. In all seroconversion panels both the ADVIA Centaur assay and the comparative assay detected the first reactive sample at the same day.

Precision

Precision was evaluated according to the National Committee for Clinical Laboratory Standards protocol EP5-A.[12] A five member panel and controls were assayed in three replicates twice a day for 20 days (n=120 for each sample). The following results were obtained using one reagent lot and a stored calibration curve.

	Mean	Within-run		Between run		Total	
Sample	S/CO	SD	CV(%)	SD	CV(%)	SD	CV(%)
Negative Control	0.13	0.01	6.0	0.01	8.3	0.01	11.0
Positive Control	1.77	0.06	3.7	0.12	6.5	0.13	7.5
K2 EDTA 1	0.20	0.01	3.6	0.00	1.8	0.02	8.8
K2 EDTA 2	0.67	0.04	5.3	0.03	4.5	0.05	7.2
K2 EDTA 3	1.24	0.06	4.9	0.06	4.5	0.09	7.6
K2 EDTA 4	1.70	0.07	4.3	0.07	4.3	0.13	7.6
K2 EDTA 5	2.31	0.10	4.5	0.14	6.1	0.22	9.4
Lithium heparin 1*	0.16	0.00	2.8	0.00	1.8	0.01	3.7
Lithium heparin 2	0.69	0.03	4.4	0.03	3.7	0.04	6.0
Lithium heparin 3	1.32	0.08	6.0	0.06	4.6	0.10	7.6
Lithium heparin 4	1.81	0.11	5.9	0.11	5.9	0.15	8.3
Lithium heparin 5	2.49	0.21	8.2	0.14	5.5	0.25	9.9
Sodium heparin 1	0.28	0.01	4.2	0.00	1.5	0.02	7.1
Sodium heparin 2	0.72	0.04	5.6	0.02	2.3	0.05	6.5
Sodium heparin 3	1.46	0.11	7.4	0.05	3.6	0.14	9.7
Sodium heparin 4	1.98	0.14	7.0	0.12	6.3	0.20	9.9
Sodium heparin 5	2.70	0.25	9.1	0.13	4.7	0.29	10.9
Serum 1	0.18	0.02	9.4	0.01	4.5	0.02	10.4
Serum 2	0.69	0.03	4.4	0.03	3.9	0.04	6.5
Serum 3	1.22	0.08	6.2	0.02	1.8	0.08	6.9
Serum 4	1.75	0.09	4.9	0.07	4.3	0.12	6.8
Serum 5	2.52	0.12	4.9	0.13	5.2	0.18	7.3

* For this sample 117 observations were obtained due to routine laboratory error.

System Reproducibility

The ADVIA Centaur HAV IgM reproducibility study was performed at 3 testing sites utilizing 3 reagent lots per site. A 20-member panel, controls, and calibrators were assayed in replicates of 5, on a single run per day, over 6 days, for each lot. The study was completed with a single calibration of the assay (one calibration interval). Standard deviation and percent CV were calculated for within-run, between-day, and total. Replicates of negative samples (Panel member 1 and Low Control) reported as below the reportable range were non-numerical results and were excluded from the analyses.

Panel Member	Matrix or Control Lot	Mean ADVIA Centaur HAV IgM S/CO Value	Within Run[a]		Between Run[b]		Between Testing Site[c]		Between Lot[d]		Total[e]		Number of Observations
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
1	EDTA	0.09	0.01	N/A	0.00	N/A	0.01	N/A	0.04	N/A	0.04	N/A	90
1	Li Heparin	0.10	0.01	N/A	0.00	N/A	0.02	N/A	0.03	N/A	0.04	N/A	92
1	Na Heparin	0.11	0.01	N/A	0.00	N/A	0.02	N/A	0.03	N/A	0.03	N/A	93
1	Serum	0.09	0.01	N/A	0.00	N/A	0.02	N/A	0.04	N/A	0.04	N/A	91
2	EDTA	2.77	0.17	6.0	0.10	3.5	0.36	13.1	0.00	0.0	0.41	14.8	270
2	Li Heparin	2.85	0.19	6.7	0.14	5.0	0.39	13.6	0.00	0.0	0.46	16.0	270
2	Na Heparin	2.86	0.20	7.1	0.19	6.7	0.47	16.5	0.00	0.0	0.55	19.2	270
2	Serum	2.60	0.15	5.9	0.14	5.3	0.28	10.8	0.00	0.0	0.35	13.4	270
3	EDTA	1.08	0.05	4.7	0.02	2.0	0.09	8.6	0.00	0.0	0.11	10.0	270
3	Li Heparin	1.16	0.07	6.0	0.04	3.4	0.12	10.6	0.00	0.0	0.15	12.6	270
3	Na Heparin	1.16	0.08	6.6	0.05	4.7	0.13	10.8	0.00	0.0	0.16	13.5	270
3	Serum	1.01	0.06	5.5	0.04	3.7	0.08	8.1	0.00	0.0	0.11	10.5	270
4	EDTA	1.69	0.09	5.1	0.04	2.2	0.16	9.6	0.00	0.0	0.19	11.0	270
4	Li Heparin	1.84	0.09	4.7	0.08	4.5	0.23	12.5	0.00	0.0	0.26	14.1	270
4	Na Heparin	1.84	0.10	5.3	0.09	4.7	0.22	11.8	0.00	0.0	0.25	13.8	270
4	Serum	1.59	0.08	5.2	0.05	3.4	0.15	9.3	0.00	0.0	0.18	11.2	270
5	EDTA	0.54	0.03	5.8	0.02	3.7	0.06	12.0	0.00	0.0	0.07	13.9	270
5	Li Heparin	0.63	0.05	7.5	0.03	5.4	0.08	12.8	0.00	0.0	0.10	15.8	270
5	Na Heparin	0.62	0.04	6.5	0.04	6.8	0.08	12.1	0.00	0.0	0.10	15.3	270
5	Serum	0.53	0.03	5.1	0.02	3.8	0.06	11.9	0.00	0.0	0.07	13.5	270
Low Control	702034	0.08	0.00	N/A	0.00	N/A	0.02	N/A	0.03	N/A	0.03	N/A	90
High Control	702034	1.82	0.09	4.9	0.09	4.9	0.22	12.0	0.00	0.0	0.25	13.8	270

a Variability of the assay performance within day (all testing sites and reagent lots).
b Variability of the assay performance between days (all testing sites and reagent lots).
c Variability of the assay performance between testing sites (from testing site to testing site).
d Variability of the assay performance between reagent lots (from reagent lot to reagent lot, across all testing sites).
e Variability of the assay performance incorporating all testing sites, all reagent lots, and all days.
CV = coefficient of variation
NA = Not applicable

Cross-Reactivity

The ADVIA Centaur HAV IgM assay was evaluated for potential cross-reactivity with viral antibodies and disease state specimens. The nonreactive anti-HAV IgM status of each specimen was verified using a comparative anti-HAV IgM assay. The following results were obtained using the ADVIA Centaur HAV IgM assay.

		ADVIA Centaur HAV IgM Results		
Clinical Category	Number Tested	Nonreactive	Equivocal	Reactive
Hepatitis B Infection (HBV)	2	2	0	0
Hepatitis C Infection (HCV)	10	10	0	0
Epstein-Barr Virus (EBV) IgG	10	9	1	0
Epstein-Barr Virus (EBV) IgM	10	10	0	0
Herpes Simplex Virus (HSV) IgG	10	10	0	0
Herpes Simplex Virus (HSV) IgM	10	10	0	0
Syphilis IgG	10	10	0	0
Human Immunodeficiency Virus (HIV 1/2)	10	10	0	0
Varicella Zoster (VZV) IgG	10	10	0	0
Cytomegalovirus (CMV) IgG	2	2	0	0
Cytomegalovirus (CMV) IgM	3	3	0	0
Toxoplasma IgG	10	10	0	0
Toxoplasma IgM	9	9	0	0
Rubella IgG	10	10	0	0
Multiparity	10	10	0	0
Flu Vaccine Recipient	6	6	0	0
Rheumatoid Arthritis (RF)	9	9	0	0
Anti-Nuclear Antibody (ANA)	5	5	0	0
Systemic Lupus Erythematosus (SLE)	2	2	0	0
Human Anti-Mouse Antibodies (HAMA)	9	9	0	0
Total Samples Tested	157	156	1	0

Endogenous Interferents

The potentially interfering effects of hemoglobin, triglycerides, conjugated bilirubin, unconjugated bilirubin, high protein, and low protein were evaluated using 10 serum samples. Interference testing was determined according to NCCLS Document EP7-P.[13] In addition, a potentially interfering effect of biotin was evaluated using 6 plasma samples spiked with several levels of biotin.

Specimens that are . . .	Demonstrate ≤ 10% change in results up to . . .
hemolyzed	500 mg/dL of hemoglobin
Lipemic	3000 mg/dL of triglycerides
Icteric	60 mg/dL of conjugated bilirubin
Icteric	40 mg/dL of unconjugated bilirubin
Proteinemic (high)	12 g/dL of protein
Proteinemic (low)	3.5 g/dL of protein
Biotin spiked	50 ng/ml of biotin

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

References

1. Hollinger FB, Ticehurst JR. Hepatitis A virus. In: Fields BN, Knipe DM, Howley PM, Fields virology, 3rd ed. Philadelphia, PA: Lippincott-Raven Publishers, 1996:735–782.
2. Stapleton JT. Hepatitis A virus: biology, pathogenesis, epidemiology, clinical description, and diagnosis. In: Specter S, Viral hepatitis: diagnosis, therapy, and prevention; Totowa, NJ: Humana Press Inc., 1999:7–33.
3. Cuthbert JA. Hepatitis A: old and new. Clin Microbiol Rev 2001;14(1):38–58.
4. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. J Infect Dis 2000;182:12–17.
5. National Committee for Clinical Laboratory Standards. Procedures for handling and processing of blood specimens; approved guideline. NCCLS Document H18-A2. Wayne (PA):NCCLS;1999 Oct 40p.
6. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377–82, 387-8.
7. National Committee for Clinical Laboratory Standards. Protection of laboratory workers from instrument biohazards and infectious disease transmitted by blood, body fluids, and tissue; approved guideline. NCCLS Document M29-A2. Wayne (PA):NCCLS;2001 Dec 105p.
8. Federal Occupational Safety and Health Administration, Bloodborne Pathogens Standard, 29 CFR 1910.1030.
9. National Committee for Clinical Laboratory Standards. Assessment of the clinical accuracy of laboratory tests using Receiver Operator Characteristics (ROC) plots; approved guideline. NCCLS Document GP-10A. Wayne (PA): NCCLS;1995.
10. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27–33.
11. National Committee for Clinical Laboratory Standards. How to define, determine, and utilize reference intervals in the clinical laboratory; approved guideline. NCCLS Document C28-A. Wayne (PA): NCCLS;1995.
12. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices-second edition; approved guideline. NCCLS Document EP5-A. Wayne (PA): NCCLS;1995.
13. National Committee for Clinical Laboratory Standards. Interference testing in clinical chemistry; proposed guideline. NCCLS document EP7-P. Wayne (PA):NCCLS;1986.